

# Human Thymic Stromal Lymphopoietin Preferentially Stimulates Myeloid Cells<sup>1</sup>

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The sequence of a novel hemopoietic cytokine was discovered in a computational screen of genomic databases, and its homology to mouse thymic stromal lymphopoietin (TSLP) suggests that it is the human orthologue. Human TSLP is proposed to signal through a heterodimeric receptor complex that consists of a new member of the hemopoietin family termed human TSLP receptor and the IL-7R  $\alpha$ -chain. Cells transfected with both receptor subunits proliferated in response to purified, recombinant human TSLP, with induced phosphorylation of Stat3 and Stat5. Human TSLPR and IL-7R $\alpha$  are principally coexpressed on monocytes and dendritic cell populations and to a much lesser extent on various lymphoid cells. In accord, we find that human TSLP functions mainly on myeloid cells; it induces the release of T cell-attracting chemokines from monocytes and, in particular, enhances the maturation of CD11c<sup>+</sup> dendritic cells, as evidenced by the strong induction of the costimulatory molecules CD40 and CD80 and the enhanced capacity to elicit proliferation of naive T cells. *The Journal of Immunology*, 2001, 167: 336–343.

**T**he development of functional B and T lymphocytes from their immature precursor cells is in part coordinated by a network of soluble and membrane-bound factors. Among these, thymic stromal lymphopoietin (TSLP)<sup>3</sup> was first identified as an activity from the conditioned medium of a mouse thymic stromal line (1) that supported the development of B cells. The activities of mouse TSLP (mTSLP) overlap with those of IL-7; both stimulate thymocytes and mature T cells and facilitate B lymphopoiesis in cultures of fetal liver and bone marrow lymphocyte precursors (2, 3). Mouse TSLP promotes the development of B cells to the B220<sup>+</sup>/IgM<sup>+</sup> stage, whereas IL-7 supports the development of B cells to the less mature B220<sup>+</sup>/IgM<sup>+</sup> pre-B cell stage (3). The recent cloning of mTSLP cDNA revealed that the encoded protein is a member of the hemopoietic cytokine family (4–6).

As is frequently observed among structurally related members of the hemopoietic cytokine family, mTSLP and mIL-7 share a receptor subunit, the IL-7R  $\alpha$ -chain, in their respective signaling complexes, partly explaining the overlapping biological profiles of these two related cytokines (7). However, whereas the IL-7R complex requires the common  $\gamma$ -chain (R $\gamma_c$ ) in addition to IL-7R $\alpha$ , mTSLP instead recruits a TSLP-specific chain named TSLPR (7, 8) that had been previously identified as an orphan hemopoietic receptor most closely related to R $\gamma_c$  (9, 10). Mouse TSLP sequentially binds first with low affinity to TSLPR and then forms a high affinity complex with IL-7R $\alpha$  (7). Both TSLP and IL-7 induce

tyrosine phosphorylation of the transcription factor Stat5, but whereas IL-7-mediated signaling occurs via activation of Janus kinases JAK1 and JAK3, TSLP is unable to activate either enzyme (3, 11), but may instead interact with JAK2 (10). This evidence points to as yet undefined biological effects of TSLP that are not shared with IL-7. Although mouse TSLP was first reported in 1994, no human homologue of TSLP has been identified.

Here we describe a new human hemopoietic cytokine and its corresponding primary receptor, respectively called hTSLP and hTSLPR. We provide evidence that the functional receptor for hTSLP consists of hTSLPR and hIL-7R $\alpha$ , and activation of this complex leads to phosphorylation of both Stat5 and Stat3. Biological characterization indicates that hTSLP acts primarily on myeloid cells. As suggested by the coexpression profile of TSLPR and IL-7R $\alpha$  on monocytes and dendritic cells, we find that human TSLP can induce the release of T cell-attracting chemokines from monocytes and potentially enhances the T cell stimulatory capacity of the CD11c<sup>+</sup> subset of dendritic cells.

## Materials and Methods

### Cell lines

Human 293T epithelial cells were maintained in DMEM (Life Technologies, Gaithersburg, MD) supplemented with 10% FCS. The pro-B cell line Ba/F3 was maintained in RPMI 1640 (Life Technologies) supplemented with 10% FCS and 10 ng/ml mouse IL-3. QBI-293A human embryonic kidney cells used for adenovirus expression were grown in CMF-1 medium (CellWorks, San Diego, CA). BOSC23 cells were maintained in DMEM-10% FCS and guanine phosphoribosyltransferase selection reagents (Specialty Media, Lavellette, NJ). The cells were transferred to DMEM-10% FCS without guanine phosphoribosyltransferase selection reagents 2 days before transfection.

### Adenovirus expression of human TSLP and purification of the recombinant protein

The mature coding region of human TSLP (residues 29–159) was fused to the signal sequence of mouse SLAM (12) and inserted into a modified version of transfer vector pQBI-AdCMV5-GFP (Quantum Biotechnologies) by PCR. Recombinant adenovirus was produced as described in Quantum applications manual 24AL98. Recombinant virus was used to infect  $5 \times 10^8$  cells in 1 l CMF-1 with culture in a Nunc Cell Factory (Nalge Nunc, Naperville, IL) for 3 days. The culture medium was clarified

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<sup>3</sup> Abbreviations used in this paper: TSLP, thymic stromal lymphopoietin; mIL-3, murine IL-3; mTSLP, mouse TSLP; hTSLP, human TSLP; TSLPR, TSLP receptor; R $\gamma_c$ , common  $\gamma$ -chain receptor; JAK, Janus kinase; DC, dendritic cells; TARC, thymus and activation-regulated chemokine; MDC, macrophage-derived chemokine; PARC, pulmonary and activation-regulated chemokine.

by centrifugation, dialyzed, and filtered before application to a 5-ml Q-Sepharose column. The Q-Sepharose flow-through, which contained human TSLP, was loaded onto a 5-ml HiTrap heparin (Pharmacia, Uppsala, Sweden) column at 5 ml/min. The column was washed with 50 mM Tris-HCl (pH 8.0) and 1 mM EDTA and eluted with a gradient from 0 to 2.5 M NaCl in 50 mM Tris-HCl (pH 8.0) and 1 mM EDTA. The peak fractions were concentrated, dialyzed against PBS and quantitated by SDS-PAGE and Coomassie staining using lysozyme as a standard. A similar procedure was followed to prepare mouse TSLP.

#### Ba/F3 retroviral-mediated gene transfer and proliferation assays

Human IL-7 $\alpha$  cDNA and human TSLPR cDNA were cloned by PCR in the retroviral vectors pMX and its derivative pMX-puro to give pMX-hIL-7 $\alpha$  and pMX-puro-TSLPR, respectively (13). The BOSC23 packaging cell line was transiently transfected with retrovirus constructs using Fugene 6 (Life Technologies) according to the manufacturer's protocol. Retrovirus-containing supernatants were collected after 2 days. Ba/F3 cells were infected with retroviral supernatants for 48 h on petri dishes coated with 40  $\mu$ g/ml recombinant fibronectin fragments (Retronectin; Takara, Shiga, Japan). After 48 h puromycin (1  $\mu$ g/ml) was added to those cells infected with virus obtained from pMX-puro constructs. The efficiency of infection of Ba/F3 cells was >90% as assessed by parallel infection with the test construct pMXI-EGFP encoding the enhanced green-fluorescent protein (EGFP). Proliferation assays using Ba/F3 cells were performed as previously described (14). Cells were washed three times with RPMI medium and plated at a density of 5000 cells/well. Cells were grown with serial 3-fold dilutions of mouse IL-3, human and mouse TSLP, or human IL-7 (all starting concentrations of 225 ng/ml). After 36 h at 37°C, Alamar Blue REDOX indicator (Trek Diagnostic Systems, Medina, NY) was added to a final concentration of 10% (v/v) to each well. Cells were allowed to grow for 5–8 h more, after which plates were measured with a fluorometer.

#### Quantitation of mRNA expression

cDNA libraries from various tissue and cellular sources were prepared as described previously (15) and used as templates for Taqman-PCR analyses. cDNAs (50 ng/reaction) were analyzed for the expression of hTSLP, hTSLPR, and hIL7 $\alpha$  genes by the fluorogenic 5'-nuclease PCR assay (16), using an ABI Prism 7700 Sequence Detection System (Perkin-Elmer, Foster City, CA). Reactions were incubated for 2 min at 50°C, denatured for 10 min at 95°C, and subjected to 40 two-step amplification cycles with annealing/extension at 60°C for 1 min, followed by denaturation at 95°C for 15 s. The amplicons used for hTSLP, hTSLPR, and IL-7 $\alpha$  covered bp 246–315, 263–335, and 519–596, respectively (numbering starts at the start codon), and were analyzed with 6-carboxyfluorescein (FAM)-labeled probes. Values were expressed as femtograms per 50 ng total cDNA. Primers and probes for human chemokine and chemokine receptors were obtained from Perkin-Elmer as predeveloped assay reagents. Chemokine and chemokine receptor expression was adjusted for the amount of 18S rRNA and compared with the control (calibrator) samples using the comparative  $\Delta\Delta C_T$  method (17). Samples were measured in duplicate. 18S rRNA levels were determined under primer-limited conditions in multiplex reactions as recommended using a Vic-labeled probe (Perkin-Elmer).

#### Cell isolation and culture

PBMC were purified from buffy coats of healthy volunteers (Stanford Blood Bank, Palo Alto, CA) by centrifugation over Ficoll. Human monocytes were isolated from PBMC by negative depletion using anti-CD2 (Leu 5A), anti-CD3 (Leu 4), anti-CD8 (Leu 2a), anti-CD19 (Leu 12), anti-CD20 (Leu 16), anti-CD56 (Leu 19; BD PharMingen, San Jose CA), anti-CD67 (IOM 67; Immunotech, Westbrook, ME), and anti-glycophorin A (10F7 MN; American Type Culture Collection, Manassas, VA) mAbs and sheep anti-mouse IgG-coupled magnetic beads (Dyna, Oslo, Norway) as described previously (18). Monocytes were cultured in RPMI and 10% FCS at a density of  $10^6$  cells/ml in the presence or the absence of IL-7 (50 ng/ml) and/or hTSLP (50 ng/ml) for 24 h, and culture supernatants and cells were harvested for quantitation of cytokine production or gene expression analyses. Human CD11c $^+$  dendritic cells (DC) were isolated from PBMC as previously described (19). Briefly, PBMC were incubated with anti-CD3, anti-CD14, anti-CD19, and anti-CD56 mAbs and depleted from lineage-positive cells using magnetic beads (Dyna), and CD11c $^+$  lineage-negative blood DC were subsequently isolated by cell sorting to reach a purity of >99%. Freshly sorted cells were cultured in RPMI 1640 containing 10% FCS at  $5 \times 10^4$ /100  $\mu$ l in flat-bottom 96-well half-area culture plates or at  $1 \times 10^5$ /200  $\mu$ l in flat-bottom 96-well plates, with or without TSLP (15 ng/ml).

#### TARC ELISA

The production of tryms and activation-regulated chemokine (TARC)/CCL17 in culture supernatants was determined by chemokine-specific ELISA using MAB364 as capture reagent and BAF364 as detection reagent (R&D Systems, Minneapolis, MN). The sensitivity of the assay was 50 pg/ml.

#### DC viability and flow cytometric analysis

After 24 h of culture, DC were harvested and resuspended in an EDTA-containing medium to dissociate the clusters. Viable DC were first counted using trypan blue exclusion of dead cells. Remaining cells were stained with a variety of mouse anti-human FITC-conjugated mAbs including anti-HLA-DR (BD Biosciences, Mountain View, CA); anti-CD40, -CD80, and -CD86 (all from PharMingen); or an Ig-G1 isotype control (BD Biosciences) and were analyzed with a FACScan flow cytometer (BD Biosciences). Dead cells were excluded based on side and forward scatter characteristics.

#### T cell proliferation assay

Naive CD4 $^+$ /CD45CD45RA $^+$  T cells were isolated from adult blood buffy coats by negative depletion of cells expressing CD14, CD19, CD56, CD8, CD45RO, HLA-DR, and glycophorin A using magnetic beads (Dyna). More than 95% of the purified cells had the CD4 $^+$ CD45RA $^+$  naive T cell phenotype. CD11c $^+$  DC were washed twice to remove any cytokine and cocultured with  $5 \times 10^4$  allogeneic naive CD4 $^+$  T cells in round-bottom 96-well culture plates at increasing DC/T cell ratios. All cocultures were conducted in triplicate. DC alone and T cells alone were used as controls. After 5 days cells were pulsed with 1  $\mu$ Ci [ $^3$ H]thymidine (Amersham, Arlington Heights, IL) for 16 h before harvesting and counting of radioactivity.

#### Stat3 and Stat5 activation assays

Stable Ba/F3 transfectant cells ( $\sim 2.5 \times 10^7$  cells) were starved for 4–6 h and then stimulated at  $10^6$  cells/ml for 15 min with either 10 ng/ml mL-3 or 30 ng/ml hTSLP. After stimulation cells were harvested and incubated for 15 min at 4°C in lysis buffer containing 50 mM Tris-HCl (pH 7.5), 300 mM NaCl, 2 mM EDTA, 0.875% Brij 97, 0.125% Nonidet P-40, 10 mg/ml aprotinin, 10 mg/ml leupeptin, 1 mM PMSF, 1 mM Na<sub>3</sub>VO<sub>4</sub>, and 1 mM NaF. Cell lysates were clarified by centrifugation at  $12,000 \times g$  for 15 min, and supernatants were subjected to 8% SDS-PAGE. Proteins were electrotransferred onto nylon membranes (Immobilon-P, Millipore, Bedford, MA) and detected by Western blot analysis using rabbit Abs against anti-phospho-Stat3 and anti-phospho-Stat5 (New England Biolabs, Beverly, MA) or anti-Stat3 and anti-Stat5 (Santa Cruz Biotechnology, Santa Cruz, CA), followed by mouse anti-rabbit Ig HRP. Immunoreactive bands were visualized with ECL (SuperSignal West Dura Extended Duration Substrate, Pierce, Rockford, IL) on ECL film (Eastman Kodak, Rochester, NY). For reprobing blots were stripped with 200 mM glycine and 1% SDS, pH 2.5, for 30 min at 65°C.

## Results

#### Human TSLP was identified computationally

Genomic databases were searched with a computationally derived sequence profile of the IL-7 helical cytokine family using methods described by Oppmann et al. (20). This search identified an expressed sequence tag (GenBank accession no. AA889581) encoding an almost full-length novel human cytokine. A search across a panel of cDNA libraries of various tissues and cell lines identified the lung fibroblast sarcoma cell line MRC5 as a source for a full-length clone of this novel cytokine. The open-reading frame (Fig. 1A) encodes a 159-residue polypeptide that includes a 28-amino acid signal sequence. This novel human cytokine most likely represents the human orthologue of mTSLP (Fig. 1C), although the low sequence identity between the two proteins (43%) is reminiscent of the great divergence between human and mouse IL-3 (21). The N-terminal hTSLP residue was determined to be Y29, so the mature sequence (residues 29–159) encodes a protein with calculated m.w. of 14.9 kDa with six cysteine residues and two N-glycosylation sites. Human TSLP was expressed and purified from adenovirus hTSLP-infected 293 cells and analyzed on SDS gels (data not shown); the purified protein has a  $M_r$  of 23 kDa, indicating that the mature protein is glycosylated. Quantitative PCR

**A**

TSLP\_HUMAN/(159)1-159 MFPPFALLYVLSVSFRKIFILQ-LVGLVLIYDFITNCDPEKIKAAVLSTISKDITTYMSTGTSSTERNNTVSCSNRRHCITETQS  
 TSLP\_MOUSE/(140)1-140 -----MVLRLSLFIIQVLRMGLEYNFSSGNGTSTTKITCNIIHFHDTQDLKGA---EQIEDGESKAGALKHEY  
 SS Leader sequence HELIX A HELIX B

TSLP\_HUMAN/(159)1-159 LIFNPITACCASTAKEMFAMKKAALATWCPCYSEIOTATQAMKKRRKRKVTNKCEQVSLQGLWRRNRRPLKQO  
 TSLP\_MOUSE/(140)1-140 YLNPIPCCPSIPDKTARRRREALNDHCPGYPERN---DGTQEMAEVQNICLNGTSQILRLWYSQMOSPE---  
 SS HELIX C HELIX D

**B**

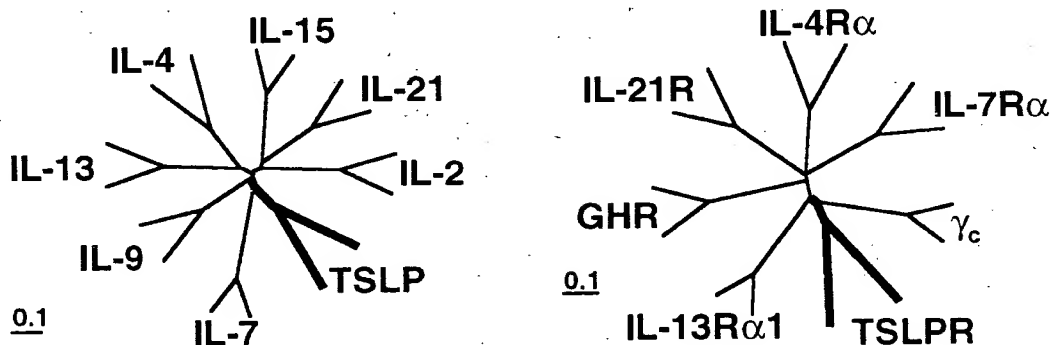
TSLPR\_HUMAN/1-371 MGRLLVLLWGAAVFLGGWMAAGQGAAAEQV---QIQITFYFNLEIVQVTWN-A-SKYSRTNITFHYEFNCDAYDQCTNYLTQE  
 TSLPR\_MOUSE/1-370 -----MAWAIAVILIPRLIAAA AAAAAAVTSRQDVTVCCHOETVEVFWGSGPDHSAITISLEFVYCTCALGPCPRVFIISG  
 Leader sequence

TSLPR\_HUMAN/1-371 -GHISGCLIDAEQRDDHITYFSITNG-THPVETA'SRWMVYVLPKPS'PKHVR'FSWHOD-AVIVTCSDSLQGLLYEVYRSPFD  
 TSLPR\_MOUSE/1-370 AGVTSGCCITPA-ADAIGLIEELALIDGGGAMVYKARQASAWKPRPDWNVTLLWTPDGDVTSWPAISYLGIDYEVYRSPFD

TSLPR\_HUMAN/1-371 T--EWDISKQENTCNVIREGLDAEKCYSEWVRVKAIMEDVYGPDTYPSDWSEVTCWQRGEIR-----DAEAEITHTPK  
 TSLPR\_MOUSE/1-370 DEDAWQTTSGPCCDLVVGGLDPAFCYDFRVRASPRAAHYGLEAQPSSEWTAVIRLSGAA'SAGDPCAAHLPLASGTASTAISP  
 WSXWS

TSLPR\_HUMAN/1-371 PKLSKFIILHSLSALFLMVSCULLSLWKLWVVKKFIIPSVDPKSIFFPGLFETICGNFQEWITDITNYAHLHKMAGAEQESGP  
 TSLPR\_MOUSE/1-370 ALAPPLLPFGCGLAALITFESLLLAALRLRRVKDAITPCVPPPSGSGSFPGLFEKHGNGFOAWIADACATAPPARTEEDDLHP  
 Transmembrane domain BOX1

TSLPR\_HUMAN/1-371 EEPLVQLAKTFAESPRMIDPQTEETKASQSLQLPHQPLQGGDVTITGGFTVMNDRSYVAL  
 TSLPR\_MOUSE/1-370 K----AKRVEPEGGTSLCTVPRPPSFEPRGP-----GGGAMVSVGGATFVMVGDSCVMTL

**C**

**FIGURE 1.** Human TSLP and TSLPR sequence and evolution. **A**, Alignment of protein sequences for human and mouse TSLP (GenBank accession no. AF232937). The four predicted  $\alpha$ -helices of the canonical hemopoietic cytokine fold in human and mouse TSLP are labeled A, B, C, and D. Identical residues are shaded black. **B**, The amino acid sequence of human TSLPR is shown in alignment with mTSLPR (GenBank accession no. AF201963). Identical residues are shaded black. The signal peptide and transmembrane domains are shown. The characteristic class I cytokine receptor motifs WSXWS and box 1 are boxed; the more typical WSXWS sequence is replaced by the sequence PSDWS in hTSLP and by PSEWT in mTSLP. **C**, Evolutionary dendrogram of selected short chain hemopoietic cytokines (left panel) and their receptors (human and mouse orthologues; right panel). The receptor tree is rooted by GHR (growth hormone receptor). Sequence data for human TSLP are available from GenBank under accession number AF338732. Sequence data for human TSLPR are available from GenBank under accession number AF338733.

analysis of a large panel of human cDNAs from various libraries and cultured cell lines showed that hTSLP expression of a 1.3-kb message was restricted to a few lung libraries and several monocyte cell samples (data not shown).

#### Identification of a receptor for hTSLP

Mouse TSLP binds to a specific receptor named mTSLPR (7). This recently described molecule is most closely related to  $R_{\gamma_c}$ , the shared receptor present in the signaling complexes for IL-2, IL-4, IL-7, IL-9, IL-15, and possibly IL-21 (22). Both  $R_{\gamma_c}$  and mTSLPR sequences were used in a focused search for a candidate hTSLPR gene; consequently, a cDNA that encoded an orphan member of the hemopoietin receptor family was identified from a proprietary database (HGS, Rockville, MD). This cDNA, designated hTSLPR,

contains an open reading frame encoding a 371-aa protein with a single transmembrane region (Fig. 1B). Human TSLPR displays the closest identity to mTSLPR (39%) followed by  $R_{\gamma_c}$  (24%), and most likely represents the human orthologue of mTSLPR (Fig. 1C). Intriguingly, an alternatively spliced, soluble, short form of the mTSLPR has been described (10), suggesting that an analogous human molecule could serve as a secreted inhibitor of hTSLP.

#### Identification of a functional heteromeric human TSLP receptor complex

The functional receptor complex for mTSLP consists of mTSLPR and mIL-7R $\alpha$ . To test the hypothesis that the functional receptor for hTSLP consists of hTSLPR and hIL-7R $\alpha$ , Ba/F3 cells were

infected with retroviral constructs encoding the receptors. While parental Ba/F3 cells would only proliferate in response to IL-3, those cells infected with either hTSLPR or hIL-7R $\alpha$  alone showed no proliferative response when either hTSLP or hIL-7 was added (Fig. 2, A and B). However, Ba/F3 cells expressing both receptors proliferated strongly in response to hTSLP, but not at all upon addition of hIL-7 (Fig. 2C). The response was specific for hTSLP, as mTSLP was not able to induce proliferation (Fig. 2C). This finding establishes that the functional receptor for hTSLP consists of two subunits, hTSLPR and hIL-7R $\alpha$ ; furthermore, we observed no cross-reactivity between mTSLP and the hTSLP receptor com-

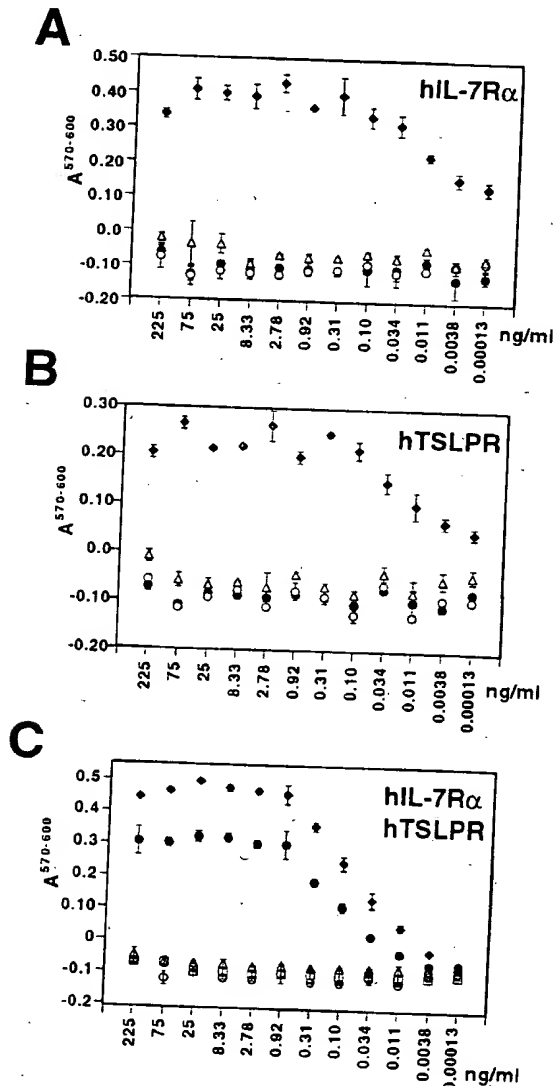
plex. The corresponding activation status of Stat5 and Stat3 was also measured in the various Ba/F3 cell populations. Fig. 3 shows that both Stat5 and Stat3 were phosphorylated upon addition of hTSLP, but only when both hTSLPR and hIL-7R $\alpha$  were present.

#### Coexpression of TSLPR and IL-7R $\alpha$ in human DC and monocytes

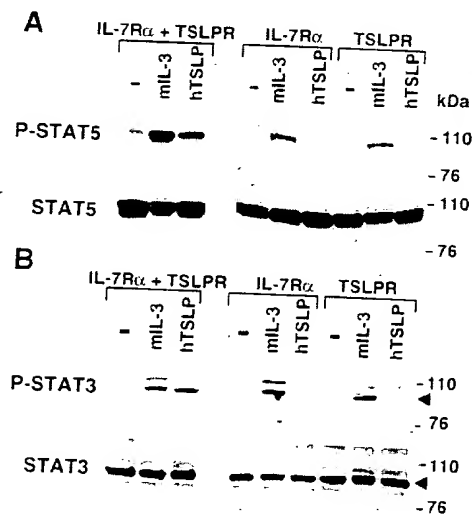
To identify possible target cells capable of responding to hTSLP we have analyzed by quantitative PCR a large panel of human cDNA libraries for the simultaneous expression of both TSLPR and IL-7R $\alpha$  mRNAs (Fig. 4). Several cell types expressed both receptors. Th cell clones, in particular activated DC, and to a lesser extent monocytes produced significant levels of both receptors, suggesting that these cell types might respond to TSLP.

#### TSLP induces TARC on freshly isolated monocyte populations and CD11c<sup>+</sup> blood DC

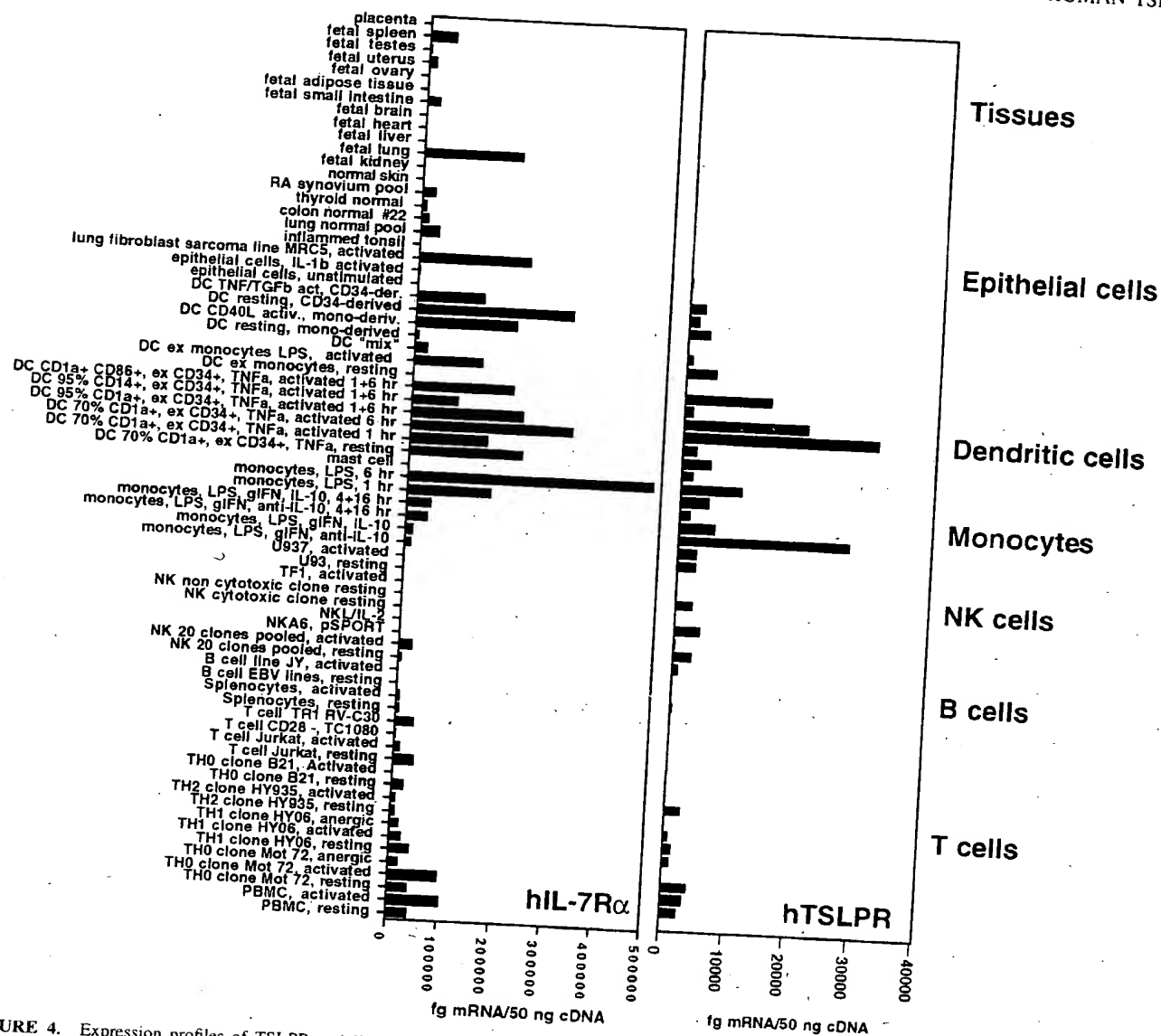
The spectrum of biological activities induced by TSLP was investigated based on the overlapping expression patterns of TSLP receptor components. cDNA was prepared from human monocytes cultured for 24 h in the presence of TSLP or IL-7, and the expression of 38 human chemokines and 20 human chemokine receptors was analyzed by quantitative real-time PCR. Interestingly, TSLP and IL-7 influenced the expression of distinct sets of chemokines (Table I), but did not affect the expression of chemokine receptors. TSLP enhanced the expression of TARC/CCL17, DC-CK1/pulmonary and activation-regulated chemokine (PARC)/CCL18, macrophage-derived chemokine (MDC)/CCL22, and MIP3 $\beta$ /CCL19. IL-7 also enhanced the expression of TARC/CCL17, MDC/CCL22, and MIP3 $\beta$ /CCL19, but, in addition, enhanced the expression of IL-8/CXCL8, CTAPIII/CXCL7, ENA78/CXCL5, and GROabg/CXCL123 and decreased the expression levels of IP-10/CXCL10, I-TACK/CXCL11, SDF1/CXCL12, MCP2/CCL8, and MCP4/CCL13 (Table I). The induction of TARC protein by TSLP on monocyte and DC populations was confirmed by ELISA. The level of TARC production by CD11c<sup>+</sup> DC was at least 10-fold higher than that by monocytes (Fig. 5).



**FIGURE 2.** TSLP signaling requires TSLPR and IL-7R $\alpha$ . Ba/F3 cells were infected with retroviruses encoding hTSLPR or hIL-7R $\alpha$  or were coinfecting with hTSLPR and hIL-7R $\alpha$  constructs. Proliferation of cells upon stimulation with either IL-7 or TSLP was determined after addition of Alamar Blue and was measured at 570–600 nm. Data represent one of three experiments with similar results. The mean of triplicate determinations is shown. The parental Ba/F3 cells proliferate only in response to mIL-3 (10 ng/ml). **A**, Ba/F3 cells expressing hIL-7R $\alpha$  do not respond to IL-7 or TSLP. **B**, Ba/F3 cells expressing hTSLPR do not respond to IL-7 or TSLP. **C**, Ba/F3 cells coexpressing hTSLPR and hIL-7R $\alpha$  proliferate in the presence of hTSLP, but not upon addition of hIL-7 or mTSLP.  $\bullet$ , mIL-3;  $\circ$ , hTSLP;  $\square$ , mTSLP;  $\triangle$ , hIL-7;  $\Delta$ , medium.



**FIGURE 3.** TSLP induces phosphorylation of Stat3 and Stat5. Ba/F3 cells expressing hIL-7R $\alpha$ , hTSLPR, or both were stimulated (see Materials and Methods) with control, mIL-3 (10 ng/ml), or hTSLP (20 ng/ml), and phosphorylation was assessed by Western blot using anti-phospho-Stat5 Ab (**A**) and anti-phospho-Stat3 Ab (**B**). Blots were reprobated using anti-Stat5 (**A**) and Stat3 Abs (**B**). Phosphorylation of Stat5 and Stat3 by hTSLP was only detected on cells expressing both receptors.



**FIGURE 4.** Expression profiles of TSLPR and IL-7R $\alpha$ . Expression of TSLPR and IL-7R $\alpha$  was measured by quantitative PCR (see *Materials and Methods*) on a panel of human (h) cDNA libraries made from various tissues and cell types. Cells were often left untreated or were treated with different stimuli as indicated before RNA was isolated. Expression levels were normalized and expressed as femtograms per 50 ng total cDNA. Coexpression of TSLPR and IL-7R $\alpha$  was detected in DC, monocytes, and, to a lesser extent, T cells.

#### TSLP activates CD11c<sup>+</sup> DC

Freshly purified immature CD11c<sup>+</sup> blood DC are known to spontaneously mature in culture (23). After 24 h in medium alone, we observed loose and irregular clumps in the DC culture (Fig. 6A). In the presence of TSLP this maturation process was dramatically enhanced. DC in culture formed tight and round clumps with fine dendrites visible at the periphery of each clump (Fig. 6B). The TSLP-induced maturation was confirmed by analyzing the surface phenotype of DC using flow cytometry. Whereas TSLP slightly up-regulated the expression of HLA-DR and CD86, it strongly induced the costimulatory molecules CD40 and CD80 (Fig. 6C). This maturation process was accompanied by an increased viability of the DC (data not shown). A titration of TSLP using log dilutions of the cytokine showed that both the effect on survival and the induction of costimulatory molecules on DC were maximal at 15 ng/ml and above and were still significant at concentrations as low as 15 pg/ml (data not shown).

We next analyzed the T cell stimulatory capacity of CD11<sup>+</sup> DC cultured 24 h in medium alone or in the presence of TSLP. DCs were cocultured with  $5 \times 10^4$  naive CD4<sup>+</sup>CD45RA<sup>+</sup> allogeneic T cells at increasing DC/T cell ratios. As assessed by [<sup>3</sup>H]thymidine incorporation on day 5 of the coculture, DC cultured with TSLP induced up to 10-fold stronger naive T cell proliferation compared with DC cultured in medium (Fig. 6D).

#### Discussion

We describe a novel human hemopoietic cytokine and its functional receptor complex. Comparison with the recently published sequence of mouse TSLP (6) suggests that the cytokine reported here is the human orthologue of mTSLP, although the degree of identity between the two proteins is low (43%). Identification of the functional hTSLP receptor complex lends further support for the evolutionary correspondence between human and mouse TSLP. As described here, the functional hTSLP receptor consists

Table 1. Effects of TSLP and IL-7 on chemokine expression<sup>a</sup>

	Media	TSLP	IL-7
CCL1	40.0	1.0	1.4
CCL2	24.8	1.0	1.5
CCL3	31.5	0.7	2.0
CCL4	28.9	1.0	1.6
CCL5	30.4	1.1	0.6
CCL7	31.3	0.7	1.4
CCL8	30.2	0.8	0.2
CCL11	40.0	1.6	1.4
CCL13	37.3	1.6	0.1
CCL14	40.0	1.3	1.3
CCL15	40.0	1.1	1.3
CCL16	40.0	2.2	7.0
CCL17	39.8	<b>195.4</b>	<b>20.1</b>
CCL18	35.8	2.9	1.7
CCL19	36.7	8.5	8.3
CCL20	40.0	1.2	1.2
CCL21	40.0	1.0	1.0
CCL22	34.3	<b>8.8</b>	<b>3.0</b>
CCL24	29.3	2.0	1.2
CCL25	40.0	1.1	1.1
CCL26	38.9	1.1	2.5
CCL27	40.0	0.8	1.2
CCL28	40.0	1.0	1.0
CXCL1-3	28.7	1.0	4.4
CXCL4	27.9	1.2	1.9
CXCL5	28.7	1.1	8.0
CXCL6	40.0	1.5	1.3
CXCL7	28.7	1.1	2.0
CXCL8	27.3	1.4	8.5
CXCL9	34.9	0.6	0.7
CXCL10	29.7	0.6	0.1
CXCL11	32.9	0.7	0.0
CXCL12	33.1	0.6	0.2
CXCL13	35.4	0.2	0.8
CXCL14	39.7	1.0	1.0
XCL1	40.0	0.9	3.8
CX3CL1	40.0	1.4	1.3

<sup>a</sup> Human monocytes were cultured in the absence or presence of hTSLP (50 ng/ml) or IL-7 (50 ng/ml) for 18 h, and expression of chemokine genes was determined by quantitative PCR. Results are expressed as 1)  $C_T$  values of nonactivated samples and 2) fold difference relative to the calibrator sample (media). Boldface values indicate significant changes.

of two subunits. The first, TSLPR, is a novel member of the hemopoietin receptor superfamily and is most closely related to the recently described mTSLPR, although both proteins have diverged substantially (39% identity). The low degree of human vs mouse sequence identity between ligands thus is mirrored in the alignment of receptor chains, consistent with the idea that these interacting molecules have coevolved (24). The closest relative of TSLPR is  $R\gamma_c$ , the shared receptor present in the signaling complexes for IL-2, IL-4, IL-7, IL-9, and IL-15. This link suggests that TSLPR is possibly a promiscuous receptor, although it cannot substitute in any of the signaling complexes that involve  $R\gamma_c$  (data not shown). The second subunit of the functional hTSLP receptor is IL-7 $\alpha$ , a chain that is also part of the functional IL-7R. Thus, the functional receptors for human and mouse TSLP both require the species-specific forms of TSLPR and IL-7 $\alpha$ . We have not observed cross-species activity of either human or mouse TSLP. In contrast, both human and mouse IL-7 show cross-species activities.

Investigation of the spectrum of biological actions induced by hTSLP was guided by the common expression patterns of TSLP receptor components. Both receptor subunits are primarily ex-

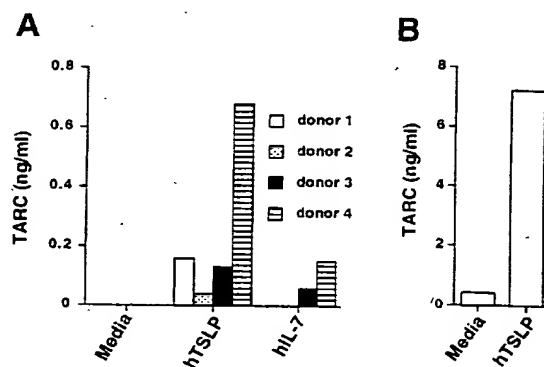


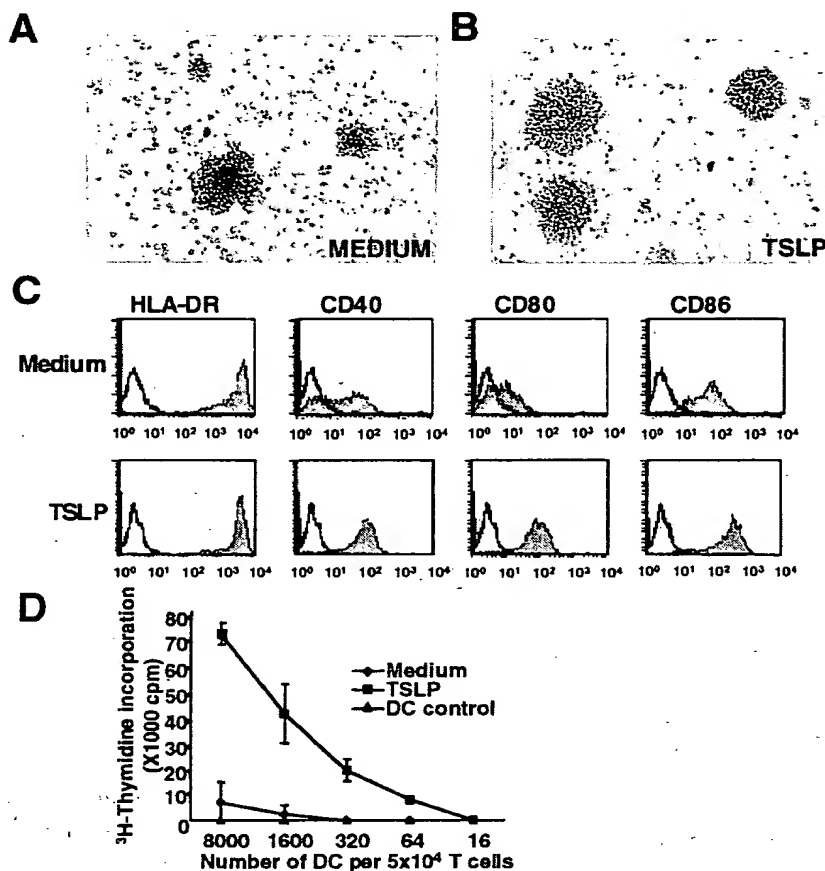
FIGURE 5. Induction of TARC by TSLP. Human monocytes (A) or CD11c<sup>+</sup> DC (B) were cultured in the absence or the presence of TSLP (50 ng/ml) or IL-7 (50 ng/ml) for 24 h, and the production of TARC was determined in the culture supernatant by ELISA.

pressed by dendritic cells and to a lesser extent by monocytes, with expression of IL-7 $\alpha$  in general approximately 10-fold higher than that of TSLPR. On freshly isolated human monocytes TSLP specifically induced the chemokines TARC, PARC, and MDC, which are all notably ligands for CCR4, a chemokine receptor predominantly found on Th2-type lymphocytes. Thus, TSLP can activate monocytes to release chemokines that may attract effector cells with a Th2 phenotype. TSLP-induced expression of TARC was very strong in the CD11c<sup>+</sup> subset of DCs. This subset, representing <1% of mononuclear cells in the blood, normally differentiates into mature DCs in response to inflammatory stimuli. The expression of TARC in these cells was accompanied by a dramatic enhancement of their maturation, as evidenced by the strong induction of the costimulatory molecules CD40 and CD80. These results suggest that this DC subset stimulated with TSLP could be a potent inducer of primary T cell-mediated immune responses. Indeed, CD11<sup>+</sup> DCs cultured in the presence of TSLP were much more potent in their capacity to elicit the proliferation of naive T cells than DC cultured in medium.

Since both mouse and human TSLP share the IL-7R  $\alpha$ -chain with IL-7, some overlap in function between these two cytokines can be expected. In mice IL-7 mediates T and B cell lymphopoiesis, and targeted deletion of IL-7 or its receptor subunits (IL-7 $\alpha$  or  $R\gamma_c$ ) leads to severe T and B lymphopenia (25–28). Both in vitro and in vivo mTSLP has been reported to costimulate thymocytes and mature T cells and to support the growth of pre-B cells, although the precise definition of the in vivo importance of mTSLP remains to be established (1, 3, 6). There is a prominent discrepancy between the function of IL-7 in mice vs humans; in the latter, IL-7 is critical for T lineage, but not B lineage, development, and either  $R\gamma_c$  or IL-7 $\alpha$  deficiency results in the arrest of only T cell development (T<sup>+</sup>B<sup>+</sup> SCID) (29–32). This argues against a major role of either hTSLP or IL-7 in human B cell development. Indeed, preliminary evidence from our laboratory suggests that hTSLP cannot support the growth of CD34<sup>+</sup> CD19<sup>+</sup> B cell precursors from normal human bone marrow either alone or in combination with IL-7. Some T cell clones, however, coexpress TSLPR and IL-7 $\alpha$  transcripts, suggesting that some T cells can respond to hTSLP. This finding is presently under investigation.

It appears from the work described here that the biological activity of TSLP, at least in humans, is not restricted to lymphoid cells. In fact, based on the prominent expression of both TSLP receptor subunits on cells of myeloid origin it is likely that TSLP mainly functions on cells of the myeloid lineages. Activities of mTSLP on these cells have not yet been reported, but the recent

**FIGURE 6.** TSLP induces the maturation of blood CD11c<sup>+</sup> DC. **A**, Culture of sorted CD11c<sup>+</sup> DC after 24 h in medium alone. DC form small and irregular clumps, with a dark center suggesting the presence of dying cells. **B**, In the presence of TSLP (15 ng/ml), CD11c<sup>+</sup> DC from the same donor form larger and round clumps with fine dendrites visible at the periphery, indicating the maturation of the DC. **C**, Surface phenotype of CD11c<sup>+</sup> DC after 24 h of culture with and without TSLP revealing the up-regulation of HLA-DR as well as the costimulatory molecules CD40, CD80, and CD86. Results shown are from one representative of four independent experiments. **D**, T cell proliferation assay using CD11c<sup>+</sup> DC matured for 24 h in medium or with TSLP (15 ng/ml) and cocultured with  $5 \times 10^4$  allogeneic CD4<sup>+</sup>CD45RA<sup>+</sup> naive T cells at increasing DC/T cell ratios. Proliferation was assessed on day 6 by measuring [<sup>3</sup>H]thymidine incorporation. CD11c<sup>+</sup> DC matured with TSLP induced up to 10-fold stronger T cell proliferation than DC matured in medium alone. Each dot represents the mean [<sup>3</sup>H]thymidine incorporation of triplicate cocultures. Vertical bars indicate the SD. DC alone ( $\Delta$ ) were used as a control and did not significantly proliferate. Results shown are from one representative of two independent experiments.



identification of a functional mTSLP-receptor complex invites a similar analysis for the present work.

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